## Detection of compounds on paper by fluorescence and phosphorescence at liquid nitrogen temperatures

We would like to call attention to a little used but valuable technique suggested by SZENT-GYÖRGYI[1]. This technique consists of the detection of substances on paper chromatograms at liquid nitrogen temperature by means of fluorescence or phosphorescence in ultraviolet light. The paper sheet is simply placed in a pyrex baking dish, cooled with a liberal quantity of liquid nitrogen, and examined with an ultraviolet light.

A Mineralight, Model V-41, Ultra-Violet Products, Inc., San Gabriel, Calif., maximum output at 2537Å, is used as the source of exciting radiation. Some compounds fluoresce strongly when directly illuminated, while other compounds can be more readily seen by means of the slowly decaying phosphorescence which persists after the lamp is turned off. The phosphorescence is accentuated by the dark background of the paper. Examples are seen in Fig. 1, 2, and 3. Since the fluorescence

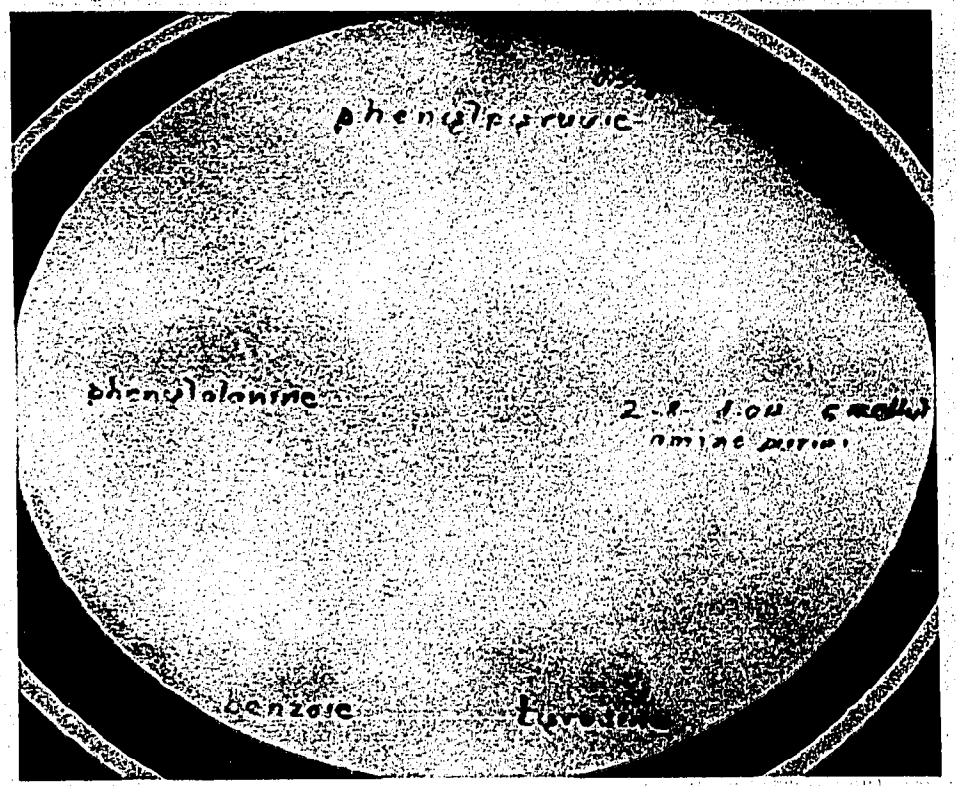


Fig. 1. Compounds photographed at room temperature with the exciting source on. The compounds, 5 μg per spot, are given in clockwise order starting at 12:00: (1) Phenylpyruvic acid; (2) 2,8-dihydroxy-6-methylaminopurine; (3) L-tyrosine; (4) benzoic acid; (5) L-phenylalanine. The film was Ansco, "Super Hypan"; a 155 mm lens at F/4, and a Wratten G filter were used. Exposure time: 15 sec. Photography was used to obtain a permanent record.

of various ionic species of a given compound can vary, exposure of the paper to ammonia or hydrochloric acid fumes may intensify the fluorescence. It is important to note that this technique is usually not applicable when the paper has been exposed to ultraviolet absorbing solvents such as phenol or pyridine.

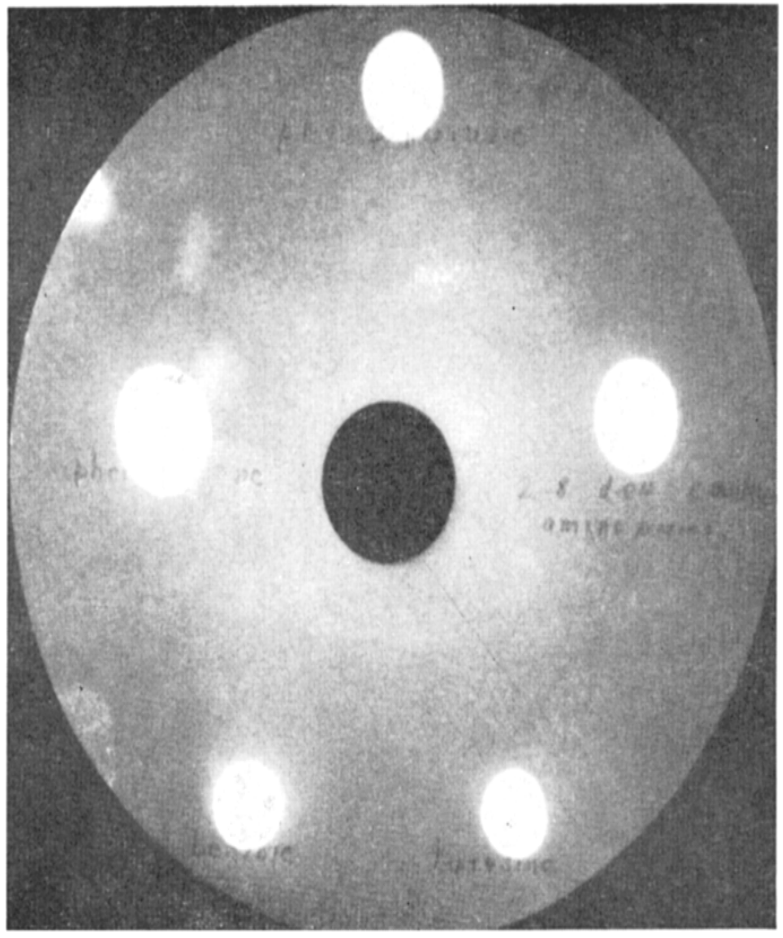

Fig. 3. Compounds photographed at liquid nitrogen temperature with the exciting source removed. No filter was used. Exposure time 40 sec. For other conditions see legend to Fig. 1. Note the intense phosphorescence of all the compounds.

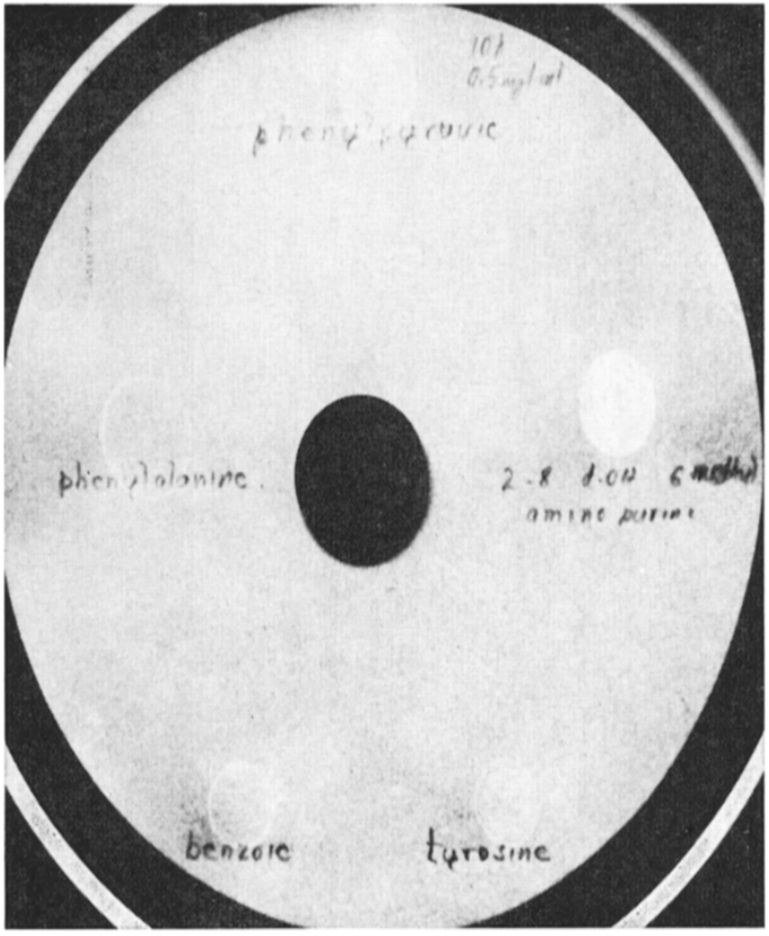


Fig. 2. Compounds photographed at liquid nitrogen temperature with the exciting source on. A Wratten G filter was used. Exposure time 30 sec. For other conditions see legend to Fig. 1. Note the intense fluorescence of the substituted purine. (The dark spot is a coin to hold the paper steady during photography.)

In our experience, any compound, including proteins, containing an aromatic ring system may be readily detected in trace amounts by these procedures. The great advantage of this procedure is that the compounds are not destroyed or altered in any way. The fluorescence and phosphorescence are easily visible with the naked eye.

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## Methyl yellow as a spray reagent in the paper chromatography of chlorinated hydrocarbon pesticides

N,N-Dimethyl-*p*-phenylazoaniline (methyl yellow) has been reported to undergo a color change when a chloroform solution of the dye is irradiated with X-rays[1]. This reaction suggested a possible application in the detection of chlorinated hydrocarbon pesticides on paper chromatograms. When the chromatograms were sprayed with a solution of methyl yellow and exposed to ultra-violet radiation, the pesticides appeared as red spots against a yellow background. Fourteen pesticides containing chlorine were tested and all were easily detected by the reagent.

The spray reagent was prepared by dissolving 100 mg of methyl yellow in 60 ml of ethanol in a 100 ml volumetric flask. Twenty-five ml deionized water was added and the solution brought to volume with ethanol. Paper chromatograms were prepared by spotting known amounts of the pesticide solution (1 $\mu$g/$\mu$l) on Whatman No. 1 chromatographic paper (8 × 8 in.). The papers were then impregnated with a 5 % solution of cottonseed oil in ethyl ether[2], and developed with pyridine–water (6:4, v/v).

Upon removal from the chromatographic tank, the chromatograms were dried in air and sprayed with the methyl yellow solution until the paper appeared uniformly wet. After being again dried in air, the sheets were finally exposed for five minutes to 30 W of ultra-violet radiation. The ultra-violet source, consisting of two 15 W 20-in. germicidal lamps in a reflector housing, was placed 2 to 4 in. above the paper chromatograms.

Minimum amounts of chlorinated pesticides which could be detected ranged from 2 to 8 $\mu$g per spot (Table I). All pesticides tested could be detected above a level of 8 $\mu$g in the chromatographic system described, the spots ranging from 5 to 10 mm in diameter. The red color formed by the methyl yellow and pesticide was quite